Three New Urea Derivatives from Pliocene-Fossil *Pinus armandii*

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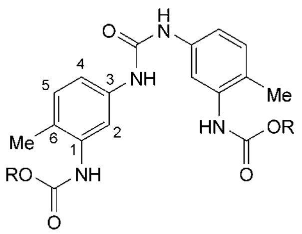
Three new urea derivatives, isolated from the Pliocene lignified wood of *Pinus armandii*, were identified as carbonylbis[imino(6-methyl-3,1-phenylene)]bis[carbamic acid] dimethyl ester (**1**), and as the corresponding dibutyl ester **2** and bis(2-methylpropyl) ester **3**. Their structures were elucidated by spectroscopic methods, including MS and 1D- and 2D-NMR techniques.

Introduction. – *Pinus armandii* FRANCHER is an economically important conifer indigenous to the southwest and central regions of China [1]. Previous chemical investigations of the heartwood of this plant led to the isolation of flavonoids and stilbenoids [2]. An interesting fact that Pliocene-lignified wood of *P. armandii* was preserved morphologically in coal mine [3] has raised questions about its phytochemical constituents. Previous investigation of fossil plants showed the presence of natural constituents [4–7]. The chemical analysis of fossil-genus *Pinus* documented series of terpenoids and their degradation products [8][9]. To probe the organic constituents of this fossil *P. armandii*, we undertook the chemical investigation of Pliocene-lignified wood of *P. armandii* collected from an open coal mine in Longlin of Yunnan Province, China. This paper describes the isolation and elucidation of three new urea derivatives, *i.e.*, of {carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester (**1**) and of the corresponding dibutyl ester **2** and bis(2-methylpropyl) ester **3**, from the MeOH extract of Pliocene-lignified wood of *P. armandii*.



1 R = Me

2 R = $MeCH_2CH_2CH_2$

3 R = Me_2CHCH_2

Results and Discussion. – Compound **1**, obtained as white powder, had a molecular formula $C_{19}H_{22}N_4O_5$ as deduced from the molecular-ion peak at *m/z* 386.1563 in the HR-EI-MS and from ^{1}H- and ^{13}C-NMR data (*Table*). Further spectral data (IR, HMQC, HMBC, ROESY, MS) could best be accommodated with the symmetrical urea

Table. *^{13}C-NMR Data* ((D$_6$)DMSO, 100 Mz) *of Compounds* **1**–**3**. δ in ppm, J in Hz.

	1	**2**	**3**
C(1)	136.7 (*s*)	136.7 (*s*)	136.8 (s)
C(2)	114.6 (*d*)	113.1 (*d*)	113.1 (*d*)
C(3)	137.9 (*s*)	138.3 (*s*)	137.4 (*s*)
C(4)	115.0 (*d*)	115.4 (*d*)	115.5 (*d*)
C(5)	130.5 (*d*)	130.3 (*d*)	130.9 (*d*)
C(6)	124.8 (*s*)	125.1 (*s*)	124.9 (*s*)
Me–C(6)	17.3 (*q*)	17.4 (*q*)	17.2 (*q*)
MeO	51.8 (*q*)	–	–
NHCONH	152.6 (*s*)	152.9 (*s*)	153.9 (*s*)
NHCOO	154.9 (*s*)	154.7 (*s*)	155.0 (*s*)
MeCH$_2$CH$_2$*C*H$_2$ or Me$_2$CH*C*H$_2$	–	64.5 (*t*)	71.8 (*t*)
MeCH$_2$*C*H$_2$CH$_2$ or Me$_2$*C*HCH$_2$	–	30.9 (*t*)	28.5 (*d*)
Me*C*H$_2$CH$_2$CH$_2$ or *Me*$_2$CHCH$_2$	–	18.9 (*t*)	18.4 (*q*)
*Me*CH$_2$CH$_2$CH$_2$	–	13.9 (*q*)	18.4 (*q*)

derivative {carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic acid] dimethyl ester (**1**).

The ^{1}H-NMR spectrum of **1** exhibited three aromatic protons at δ(H) 7.50 (*d*, $J = 2.0$ Hz), 7.10 (*dd*, $J = 2.0$, 8.3 Hz), and 7.05 (*d*, $J = 8.3$ Hz) indicating the typical trisubstituted aromatic moiety, a Me group at δ(H) 2.10 (*s*), and a MeO group at δ(H) 3.60 (*s*), the latter two being correlated to the signals at δ(C) 17.3 and 51.8 (HMQC). Two signals at δ(H) 8.80 (*s*) and 8.50 (*s*) suggested the presence of two different NH groups in **1**, which were supported by the IR absorptions at 3390 and 3278 cm^{-1}. The HMBC correlations of δ(H) 8.80 (NH) with δ(C) 114.6 (C(2)) and 124.8 (C(6)) and of δ(H) 8.50 (NH) with δ(C) 114.6 (C(2)) and 115.0 (C(4)) showed that the two NH groups were attached to the aromatic ring (*Fig.*). From the ^{13}C-NMR and DEPT spectrum, 10 C-atoms were assigned to be 2 carbonyl groups (δ(C) 154.9 and 152.6) corresponding to the IR absorptions at 1706 and 1674 cm^{-1}, 6 aromatic C-atoms, 1 Me group (δ(C) 17.3), and 1 MeO group (δ(C) 51.8). The two carbonyl groups were adjacent to the two NH groups as established by analysis of the chemical shifts and the HMBC plot. The NOE of δ(H) 3.60 (MeO) with δ(H) 8.80 (NH) in the ROESY plot and the correlation of δ(H) 3.60 with δ(C) 154.9 in the HMBC plot revealed the presence of a partial structure NHCOOMe. There were other unsaturated moieties in **1** according to the molecular formula and degree of unsaturation ($n = 11$).

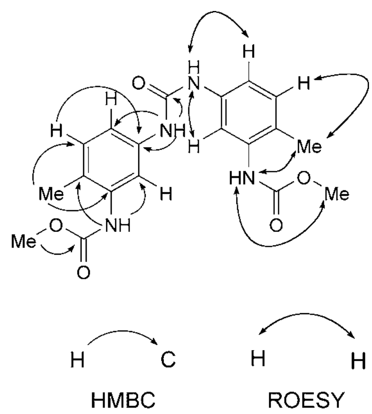
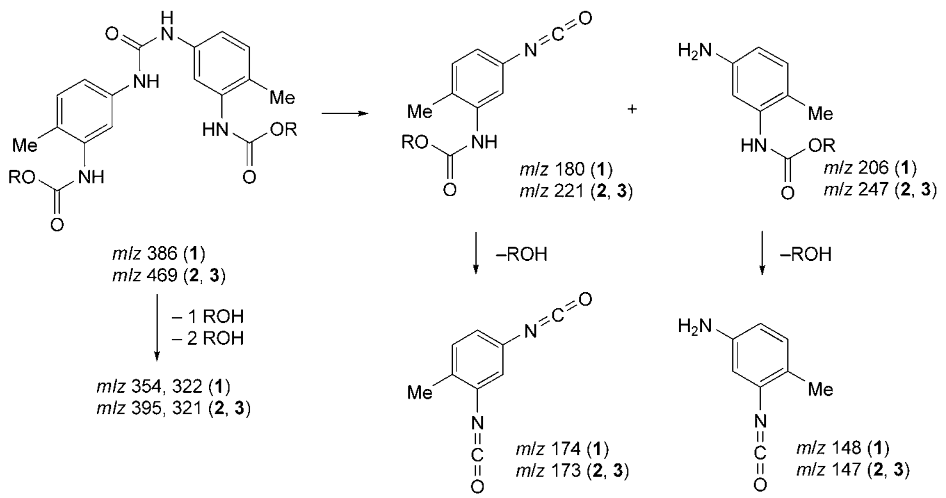


Fig. 1. *Selected HMBC and ROESY correlations of* **1**

The symmetrical urea structure of **1** was further confirmed by its MS fragmentation pattern (*Scheme*). Besides the molecular-ion peak at *m/z* 386, the EI-MS showed intense peaks at *m/z* 206 and 180 formed by the cleavage at the urea moiety, which corresponded to the ions $C_{10}H_{10}N_2O_3^+$ (by HR-ESI-MS (pos.) at *m/z* 207.0787) and $C_9H_{12}N_2O_2^+$ (by HR-ESI-MS (pos.) at *m/z* 181.0983). The weak peaks at *m/z* 354 and 322 corresponding to the ions $C_{18}H_{18}N_4O_4^+$ and $C_{17}H_{14}N_4O_3^+$ (by HR-ESI-MS (pos.) at *m/z* 355.1408 and 323.1144) were formed by loss of 1 and 2 MeOH from the molecular ion. Peaks at *m/z* 174 and 148 were due to further loss of MeOH from the ions at *m/z* 206 and 180. The configuration of **1** was further confirmed by the ROESY data revealing the NOE correlations NH (δ(H) 8.8)/MeO (δ(H) 3.60), NH (δ(H) 8.8)/Me (δ(H) 2.10), NH (δ(H) 8.5)/H−C(2), and NH (δ(H) 8.5)/H−C(4) (*Fig.*).

Scheme. *Selected Mass Fragments of* **1** (EI mode), *and of* **2** *and* **3** (FAB mode (neg.))



Compounds **2** and **3**, obtained as white powders with similar melting points as **1**, had both the molecular formula $C_{25}H_{34}N_4O_5$ as deduced from the pseudo-molecular-ion peak at *m/z* 469.2427 ($[M-H]^-$) of **2** and 469.2444 ($[M-H]^-$) of **3** in the HR-FAB-MS. Compounds **2** and **3** had the same MS cleavage pattern, similar to that of **1** (see *Scheme*), suggesting that **2** and **3** had the same skeleton structure as **1**, except for the ester group C_4H_9OOC instead of MeOOC. Based on further spectral data (^{1}H- and ^{13}C-NMR (*Table*)) and comparison with those of **1**, the structures of **2** and **3** were established as the dibutyl and bis(2-methylpropyl) ester, respectively, corresponding to the methyl ester **1**.

The ^{13}C-NMR spectra of **2** and **3** showed an additional saturated partial structure C_3H_6 as compared to **1**. Two intense peaks at *m/z* 247 and 221 formed by the cleavage at the urea moiety corresponded to the ions $C_{13}H_{15}N_2O_3^-$ (by HR-FAB-MS (neg.) at *m/z* 247.1084) and $C_{12}H_{17}N_2O_2^-$. Peaks at *m/z* 395 and 321 were formed by loss of 1 and 2 C_4H_9OH from the molecular-ion peak at *m/z* 469. The fragments at *m/z* 173 and 147, also observed in the EI-MS spectrum of **1**, were due to further loss of C_4H_9OH from the ions at *m/z* 247 and 221.

In the ^{1}H-NMR spectra, two different NH signals at δ(H) 8.91 and 8.44 for **2** and δ(H) 8.70 and 8.55 for **3** were observed. The typical trisubstituted aromatic moiety was determined by the aromatic-proton signals at δ(H) 7.70 (*d*, *J* = 2.3 Hz), 7.26 (*dd*, *J* = 2.3, 8.3 Hz), and 7.13 (*d*, *J* = 8.3 Hz) for **2** and δ(H) 7.69 (*d*, *J* = 1.8 Hz), 7.23 (*dd*, *J* = 1.8, 8.8 Hz), and 7.17 (*d*, *J* = 8.8 Hz) for **3**. The signal for the Me group at the aromatic ring was observed at δ(H) 2.30 (**2**) and 2.29 (**3**). The ^{13}C-NMR spectra of **2** and **3** were analogous to that of **1**, except for the signals of an additional saturated structure C_3H_6. The CH_2 signal at δ(C) 64.5 (**2**) and 71.8 (**3**) was assigned to the ester moiety CH_2OOC. There were 2 more CH_2 groups in **2** and 1 CH group in **3** as suggested by the

DEPT spectra. Thus, the partial structure C_4H_9O was assigned to be $MeCH_2CH_2CH_2O$ in **2** and Me_2CHCH_2O in **3**.

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Experimental Part

General. CC = Column chromatography. M.p.: uncorrected; *XRC-1* apparatus. Optical rotations: *Jasco DIP-370* digital polarimeter. UV Spectra: *UV-210A* spectrometer; in nm. IR Spectra: *Bio-Rad FTS-135* IR spectrophotometer; KBr pellets; in cm^{-1}. NMR Spectra: *Bruker AM-400* and *DRX-500* instruments; (D_6)DMSO solns. with $SiMe_4$ as internal standard; chemical shifts δ in ppm and coupling constants J in Hz. MS: *VG Auto-Spec-3000* spectrometer; in m/z (rel. %).

Plant Material. The Pliocene-lignified wood of *Pinus armandii* was collected from an open coal mine in Longling (24°42′N: 98°48′E) of Yunnan Province, People's Republic of China. The identity of the Pliocene-lignified wood material was verified by Prof. *Cheng-Sen Li*, and a voucher specimen (YN-Pliocene 1–7) has been deposited in the Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation. The powdered Pliocene-lignified wood (1.65 kg) was extracted with MeOH (3 × 6) and filtered. The filtrate was evaporated and this residue extracted with AcOEt. The AcOEt part was evaporated to give 65.9 g of a residue, which was subjected to CC (silica gel (200–300 mesh), petroleum ether/acetone 3:1): *Fractions 1–5*. *Fr. 1* (5.2 g) was further purified by repeated CC (silica gel, petroleum ether/acetone 4:1 and 3:1) and then CC (*Sephadex LH-20*): **1** (200 mg), **2** (6 mg), and **3** (7 mg).

{Carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic Acid] Dimethyl Ester (**1**): White powder. M.p. 152–154°. UV (MeOH): 258 (4.58), 216 (4.57). IR (KBr): 3390, 3278, 3129, 2955, 1706, 1674, 1607, 1535, 1452, 1424, 1356, 1309, 1257, 1226, 1128, 1075, 1062, 1001, 881, 819, 799, 775, 734, 671, 650. ^{1}H-NMR (400 MHz): 8.80 (*s*, 2 NHCOO); 8.50 (*s*, NHCONH); 7.50 (*d*, J = 2.0, 2 H, H–C(2)); 7.10 (*dd*, J = 2.0, 8.3, 2 H, H–C(4)); 7.05 (*d*, J = 8.3, 2 H, H–C(5)); 3.60 (*s*, 2 MeO); 2.10 (*s*, 6 H, Me–C(6)). ^{13}C-NMR: *Table*. EI-MS: 386 (2, M^+), 354 (2, $[M - MeOH]^+$), 322 (2, $[M - 2\,MeOH]^+$), 248 (5), 206 (62, $[M - C_9H_{12}N_2O_2]^+$), 180 (66, $[M - C_{10}H_{10}N_2O_3]^+$), 174 (33, $[M - C_9H_{12}N_2O_2 - MeOH]^+$), 148 (62, $[M - C_{10}H_{10}N_2O_3 - MeOH]^+$), 147 (100), 132 (9), 121 (42), 106 (17), 93 (19), 77 (26). HR-EI-MS: 386.1563 ($C_{19}H_{22}N_4O_5^+$; calc. 386.1590).

{Carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic Acid] Dibutyl Ester (**2**). White powder. M.p. 153–155°. ^{1}H-NMR (400 MHz): 8.91 (*s*, 2 NHCOO); 8.44 (*s*, NHCONH); 7.70 (*d*, J = 2.3, 2 H, H–C(2)); 7.26 (*dd*, J = 2.3, 8.3, 2 H, 2 H–C(4)); 7.13 (*d*, J = 8.3, 2 H, H–C(5)); 3.96 (*t*, J = 6.3, 2 $MeCH_2CH_2CH_2O$); 2.30 (*s*, 6 H, Me–C(6)); 1.72 (*m*, 2 $MeCH_2CH_2CH_2O$); 1.49 (*m*, 2 $MeCH_2CH_2CH_2O$); 1.02 (*t*, J = 4.1, 2 $MeCH_2CH_2CH_2O$). ^{13}C-NMR: *Table*. FAB-MS (neg.): 469 (100, $[M - H]^-$), 395 (12, $[M - H - C_4H_9OH]^-$), 369 (5), 321 (5, $[M - H - 2\,C_4H_9OH]^-$), 297 (4), 264 (5), 247 (92, $[M - H - C_{12}H_{18}N_2O_2]^-$), 221 (30, $[M - H - C_{13}H_{16}N_2O_3]^-$), 173 (40, $[M - H - C_{12}H_{18}N_2O_2 - C_4H_9OH]^-$), 147 (67, $[M - H - C_{13}H_{16}N_2O_3 - C_4H_9OH]^-$), 127 (6), 97 (6), 80 (5). HR-FAB-MS (neg.): 469.2427 ($C_{25}H_{33}N_4O_5^-$; calc. 469.2450).

{Carbonylbis[imino(6-methyl-3,1-phenylene)]}bis[carbamic Acid Bis(2-methylpropyl) Ester (**3**). White powder. M.p. 153–155°. ^{1}H-NMR (400 MHz): 8.70 (*s*, 2 NHCOO); 8.55 (*s*, NHCONH); 7.69 (*d*, J = 1.8, 2 H, H–C(2)); 7.23 (*dd*, J = 1.8, 8.8, 2 H, H–C(4)); 7.17 (*d*, J = 8.8, 2 H, H–C(5)); 4.03 (*d*, J = 6.8, 2 Me_2CHCH_2O); 2.29 (*s*, 6 H, Me–C(6)); 2.09 (*m*, 2 Me_2CHCH_2O); 1.11 (12 H, *d*, J = 6.2, 2 Me_2CHCH_2O). ^{13}C-NMR: *Table*. FAB-MS (neg.): 469 (20, $[M - H]^-$), 395 (10, $[M - H - C_4H_9OH]^-$), 369 (2), 321 (2, $[M - H - 2\,C_4H_9OH]^-$), 297 (3), 264 (2), 247 (100, $[M - H - C_{12}H_{18}N_2O_2]^-$), 221 (53, $[M - H - C_{13}H_{16}N_2O_3]^-$), 173 (72, $[M - H - C_{12}H_{18}N_2O_2 - C_4H_9OH]^-$), 147 (85, $[M - H - C_{13}H_{16}N_2O_3 - C_4H_9OH]^-$), 121 (7), 97 (10). HR-FAB-MS (neg.): 469.2444 ($C_{25}H_{33}N_4O_5^-$; calc. 469.2450).

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